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Methods and Devices for Local Drug Delivery in Coronary and Peripheral Arteries

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New developments in catheter design, molecular biology, and polymer chemistry have made it possible to deliver pharmaceutical agents and genetic material directly into the arterial wall to modulate the response to injury. Several local drug delivery catheters of various designs in addition to biodegradable and coated stents are currently being evaluated as devices to facilitate local delivery of agents into the arterial wall. Approaches to locally sustained delivery include the controlled release of medications, the affinity-based delivery of medications administered systemically but accumulated locally, and gene therapy. (Trends Cardiovasc Med 1993;3:163–170)

Restenosis follows percutaneous transluminal angioplasty (PTA) in 35%–50% of coronary (Nobuyoshi et al. 1988, Serruys et al. 1988) and in 19%–40% of peripheral lesions (Becker et al. 1989, Dorros et al. 1990) and results from a complex sequence of cellular responses following mechanical injury of the arterial wall. Angioplasty leads to vessel wall dissection and the resulting channels are layered with thrombus (Potkin and Roberts 1988). Smooth muscle cellular (SMC) proliferation, migration of cells from the media into the neointima or neighboring thrombus, and extracellular matrix production by the expanded cell population are key elements in the process of restenosis. Much of our current conceptualization has evolved over more than a decade of investigation of the normal repair response of the vessel wall to injury (Clowes et al. 1983a). A fundamental premise underlying the search for agents that will prevent or ameliorate restenosis is that the proliferation of SMCs is a central control point or rate-limiting step in the repair process (Schwartz et al. 1986). Thus, different kinds of antiproliferative strategies for SMCs are being evaluated to prevent restenosis. On the other hand, it seems evident that alternative strategies might be exploited, including inhibition of cellular migration, attenuation of extracellular matrix formation, and prevention of intramural dissection planes with attendant thrombosis.

The local delivery of medications to prevent restenosis could facilitate the pharmacologic strategy. Several clinical trials evaluating agents given orally have failed to demonstrate a significant reduction in the incidence of restenosis. These failures and the remarkable improvements in intravascular devices that have enabled greater access to and manipulation of atherosclerotic plaques have stimulated interest in the idea of delivering medications directly into the site of angioplasty. Immediate advantages of local therapy include enhanced specificity and reduction in the risk of systemic side effects of the agent delivered.

In addition, local delivery of pharmacologic agents may prove useful in the catheter-based treatment of acute thrombus formation in the setting of unstable angina or acute myocardial infarction or the modification of complex atherosclerotic lesions possessing a high likelihood of subsequent plaque rupture and thrombotic closure of the vessel. Nonetheless, the majority of scientific work has evaluated local drug delivery to deliver agents to modulate SMC proliferation after angioplasty in the treatment of restenosis and, thus, is the focus of this review. Selected aspects of local drug delivery including devices and general approaches are discussed.

• Catheters for Local Delivery

Aqueous or particulate matter can be deposited in the arterial wall with the use of several catheter configurations (Figure 1): (1) the double-balloon catheter (Goldman et al. 1987, Kerenyi et al. 1988), (2) the porous balloon (Wolinsky and Thung 1990), (3) a hydrogel-coated balloon catheter (Fram et al. 1992, Nunes et al. 1992), (4) a catheter containing a balloon within a porous balloon (Hong et al. 1992), (5) a porous balloon over stent (Wilensky et al. 1993), and (6) a microporous balloon catheter (Lambert et al. 1992). Other designs representing variations of these basic configurations are likely to be developed. Iontophoretic facilitated diffusion utilizing an electrical catheter propelling charged ions into the arterial wall is a theoretical method of local delivery as yet untested in vivo.

Local drug delivery catheters may be encumbered by specific problems that limit utility, including (a) expulsion of contents down arterial side branches, (b) arterial wall injury including dissection, and (c) downstreaming of contents during delivery. The double-balloon catheter is relatively cumbersome in the coronary circulation owing to the size of the open chamber created for drug delivery. Side branches from epicardial coronary arteries occur every 2–4 mm of length, making "leaks" common, although this problem may occur less frequently in large peripheral vessels. Additional in-

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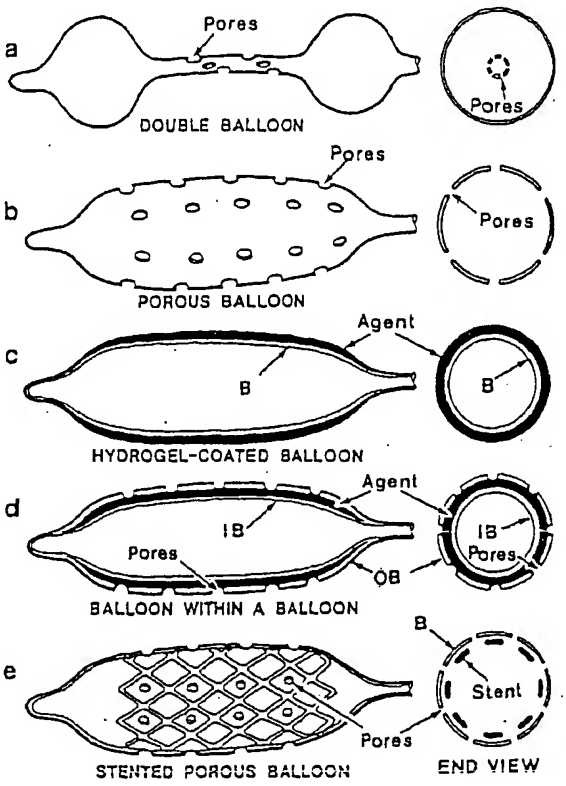


Figure 1. Schematic representation of the various balloon catheters currently utilized for local delivery in the arterial wall. (a) Double-balloon catheter. The proximal and distal balloons are inflated to form a space within the arterial lumen. Blood can be replaced by other aqueous media via the central pores through which hydrostatic pressure may also be applied for intramural infusion. (b) Porous balloon catheter. Agents can be infused through a proximal port and exit via micropores (25–75 μm) in the balloon. Hydrostatic pressure serves both to inflate the balloon and to force the balloon contents into the arterial wall. (c) Hydrogel-coated balloon. The hydrogel, which is covalently attached to the balloon, is a highly absorbent material that acts like a sponge. It can be preloaded with an aqueous solution of medication. Inflation of the balloon "squeezes" the contents of the hydrogel into the arterial wall. (d) Balloon within a porous balloon. The inner balloon (*IB*) is used to inflate the device and the outer balloon (*OB*) is porous for infusion of medication. This design enables separation of inflation and infusion. (e) Balloon over stent. The inner core is a metallic stent that can be expanded externally allowing for juxtapositioning of the outer porous balloon against the arterial wall. Medication can then be infused via the balloon. This design, like that in d, enables the mechanical and temporal separation of inflation and infusion.

jury may result from the inflation of the double balloons proximal to and distant from the angioplasty site. Downstreaming of contents occurs during expansion or deflation of porous balloons or as a result of inadequate fit between the arterial wall and the catheter. To prevent this problem, it is necessary to separate two mechanical events: (a) expansion of the drug delivery balloon to obtain satisfactory juxtapositioning against the arterial wall and (b) application of pressure to force the medication into the wall. The balloon within a porous balloon and balloon-over-stent configurations separate balloon expansion from drug delivery by enabling the porous balloon to be positioned flush with the arterial wall before hydrostatic pressure is applied to deliver the drug. Thus, downstreaming can be minimized. All of the porous balloon devices have the potential to injure the arterial wall as a result of high-velocity "jetting" of contents through one or more pores, which is most prominent when high-pressure jets are forced through pores of small diameter. This occurs if obstructed pores suddenly regain function at high delivery pressures, resulting in the explosive release of fluid. In addition to local injury, high-velocity streams of medication can also extend angioplasty-induced vascular wall dissections. This problem is minimized with the hydrogel-coated catheter, which "squeezes" its contents into the arterial wall, or with the microporous balloon catheter, which may have less capacity for "jetting" of contents. The microporous balloon is designed to reduce jetting by increasing the number of pores in the balloon while decreasing the individual pore size. The pressure drop-off through the catheter is high and oozing of the catheter's contents results. The hydrogel-coated balloon also offers the advantage of simultaneous angioplasty during intramural injection whereas the other balloon catheters are designed for use after angioplasty. In addition, the balloon-over-stent catheter delivers its contents at a lower velocity and also "oozes" its contents into the vessel wall.

Although the optimal injection pressure or volume necessary for efficient intramural deposition of pharmacologic or genetic agents has not been determined, increased levels of gene expression have been observed when a lower volume (4 mL) and a higher pressure (8 atm) were used to deliver a DNA–lipofectin complex (Mazur et al. 1993). However, Santoian et al. (1991) showed that there were increased medial damage and internal elastic lamina fragmentation, acutely, and increased intimal hyperplasia 2 weeks after local delivery of 2–5 mL of fluid delivered at 5 and 10 rather than 2 atm of pressure by a standard porous balloon catheter. We have not observed a relationship between the efficiency of intramural deposition and injection pressure in the atherosclerotic rabbit femoral artery model by using either the standard porous balloon or the balloon-over-stent catheter (unpublished results). The optimal volume of a delivered agent has also not been determined and may depend on the agent used. Unfortunately, the actual intramural deposition (that is, in the intimal and medial layers) may represent <1% of the total deposited volume (Wilensky et al. 1992 and 1993).

- Stents for Local Drug Delivery

Several types of indwelling stents have also been deployed as drug delivery devices. The utility of metallic intravascular stents has historically been limited

by a high incidence of acute thrombotic occlusion. The seeding of a stent with endothelial cells expressing tissue plasminogen activator for local anticoagulation is accordingly an example of local drug delivery intended to improve long-term stent patency (Van der Giessen et al. 1988, Dichek et al. 1989). Stent placement causes intimal proliferation that may result in restenosis and, therefore, some investigators have coated the stents with agents that reduce the SMC response. Cox et al. (1992) coated a tantalum stent with heparin and methotrexate bound to a cellulose ester polymer and placed the stent into coronary arteries of nonatherosclerotic pigs. The polymer-drug combination eluted the medication over a 3-week period as determined in vitro, but no decrease in neointimal proliferation was observed 4 weeks after implantation. A limitation noted by the authors was that only 12% of the arterial luminal surface was covered by polymer.

Several investigators have designed biodegradable stents containing medications. Polylactide and polyglycolide (or copolymers of the two) can be fashioned into stents with therapeutic agents incorporated into the matrix. Currently, biodegradable stents are limited by an inhomogeneous dissolution of the polymer that may predispose them to distal embolization. In addition, since penetration of a molecule into the arterial wall depends on both diffusion and convection, a stent containing medication may be further limited by its endoluminal location. Diffusion of medication into the blood will reduce intramural delivery unless the luminal surface is modified to favor unidirectional diffusion. Finally, stents are somewhat difficult to deploy into the distal coronary vasculature, although this should not pose a problem in the peripheral vasculature.

Inflammation of the vascular wall resulting from the placement of a foreign body is of concern since several investigators have shown an extensive arterial inflammatory response in the area of the stent arterial wall interface (Murphy et al. 1992, Lincoff et al. 1992 and 1993, Holmes et al. 1993). The inflammatory response consists of a chronic foreign-body response manifested by giant cell formation, eosinophilic and mononuclear infiltration, and medial necrosis (Murphy et al. 1992, Lincoff et al. 1992, Holmes et al. 1993). Increased neointimal proliferation caused by the stent has been observed (Murphy et al. 1992).

On the other hand, Chapman and colleagues (1990) observed no significant inflammatory response or thrombosis after deployment of a biodegradable stent into nonatherosclerotic canine femoral arteries. The difference may reflect the animal model used (canine vs swine) or the composition of the stent, since the Duke biodegradable stent is composed of L-polylactide alone (Chapman et al. 1990) whereas the other stents are composed either of polyethylene terephthalate (Murphy et al. 1992), polyglycolic/polylactic acid, polycaprolactone, polyhydroxybutyrate valerate (Lincoff et al. 1992), or polyorthoester or polyethylene oxide/polybutylene terephthalate (Lincoff et al. 1993). An exogenous fibrin stent appears to elicit less inflammation than do the polyethylene terephthalate or polyurethane stents (Holmes et al. 1993). A variation of these methods involves endoluminal paving (Slepian 1990) in which polymeric substances containing agents are delivered into the artery in a warm liquid form that subsequently cools and hardens, paving and sealing the endoluminal surface. Agents contained in the polymer matrix could presumably be released in a controlled fashion over weeks to months after endoluminal sealing. In addition, a laser balloon angioplasty (LBA) catheter method has been used as the basis for an endoluminal drug delivery strategy (McMath et al. 1990). Using this method, albumin microparticles containing heparin have been infused from a proximal port along the arterial wall, after which thermal energy from the laser-heated angioplasty balloon has been applied to "fix" the microparticles to form an endoluminal matrix. The heparin subsequently diffuses from this albumin matrix into the vascular wall. The limitations to these approaches include an as yet unknown efficiency of delivery as well as the potential for downstreaming and focal delivery of albumin microparticles.

THE EFFICACY OF AGENTS DELIVERED LOCALLY BY ANY OF THE AVAILABLE MEANS DEPENDS UPON SEVERAL FACTORS: POTENCY AND RESIDENCE TIME OF THE AGENT, LOCAL CONCENTRATION, LOCATION OF DELIVERY, AND FLUID FLUX THROUGH THE VASCULAR WALL.

• Mechanisms of Drug Disposition

The efficacy of agents delivered locally by any of the available means depends upon several factors: potency and residence time of the agent, local concentration, location of delivery, and fluid flux through the vascular wall. Major forces driving the movement of small molecules and bulk materials through the arterial wall are convection and diffusion as modified by barrier effects imposed by, for example, the endothelium or elastic laminae and by any specific affinity for elements comprising the wall. Transmural arterial fluid flux is increased when the endothelial layer is absent, for example, following angioplasty (Lin et al. 1990). Moreover, the presence of hypercholesterolemia or atherosclerosis enhances transmural flux of fluid and macromolecules (Baldwin et al. 1993). Another factor that is likely to modulate the processes of convection and diffusion is the presence and extent of vasa vasorum that may increase the intramural surface area for drug movement via diffusion or convection. This may be especially important as vasa vasorum are increased in human atherosclerotic coronary arteries (Barger et al. 1984, Zamir and Silver 1985).

Although convective forces operate in a lumen-to-adventitia direction, molecules can diffuse in all directions and the diffusion can be influenced primarily by local concentration and intrinsic properties of the molecule (that is, the diffusion coefficient). Current data suggest that many different kinds of molecules can be infused into the arterial wall, including proteins such as horseradish peroxidase (Goldman et al. 1987), carbohydrates such as heparin (Wolinsky and Thung 1990), and small molecules such as colchicine (Wilensky et al. 1992) and methotrexate (Muller et al. 1992). Agents infused locally, however, may not be retained and this constitutes a serious limitation to efficacy. For example, heparin administered via the porous balloon catheter has a residence time of ≤48 h (Wolinsky and Thung 1990). More than 90% of deposited colchicine (Wilensky et

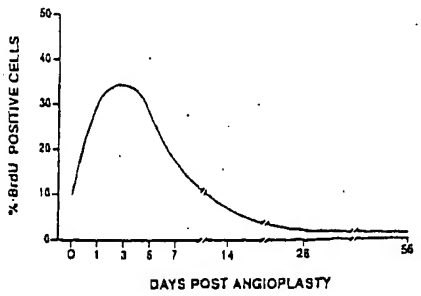


Figure 2. Time course of DNA synthesis in cells of the arterial wall after angioplasty of atherosclerotic rabbit femoral arteries. In this example, DNA synthesis was determined by pulse labeling cells via injection of the thymidine analogue, bromodeoxyuridine (*BrdU*), into rabbits. The rabbits were killed and representative arterial segments were immunostained with an antibody to BrdU. BrdU-positive cells were counted in all layers of the artery wall.

al. 1992) and methotrexate (Muller et al. 1992) are cleared in a few hours following intramural injection. Since inhibition of SMC proliferation is a major strategy for prevention of restenosis, an agent must be effective over the time period following injury during which cellular proliferation occurs. Figure 2 shows a typical time course of SMC proliferation after balloon angioplasty of the atherosclerotic rabbit femoral artery. Similar results have been obtained in several animal models, including the nonatherosclerotic but injured rat carotid artery (Clowes et al. 1983b), the atherosclerotic rabbit carotid artery (Hanke et al. 1990), and the pig carotid artery (Steele et al. 1985). Clinically symptomatic restenosis generally occurs within the first 3–6 months after coronary angioplasty (Nobuyoshi et al. 1988, Serruys et al. 1988). Accordingly, to modulate the more complex process of restenosis in human disease, an agent may need to be retained in the arterial wall for several months unless early cellular processes responsible for proliferation are irreversibly blocked.

• Approaches to Locally Sustained Drug Delivery

The problems of retention and time-controlled release of medication can be addressed by at least three approaches: (a) incorporation of the medication into a matrix that can be deposited locally (controlled release), (b) use of agents that have a specific affinity for the arterial wall (affinity-based delivery), or (c) gene therapy.

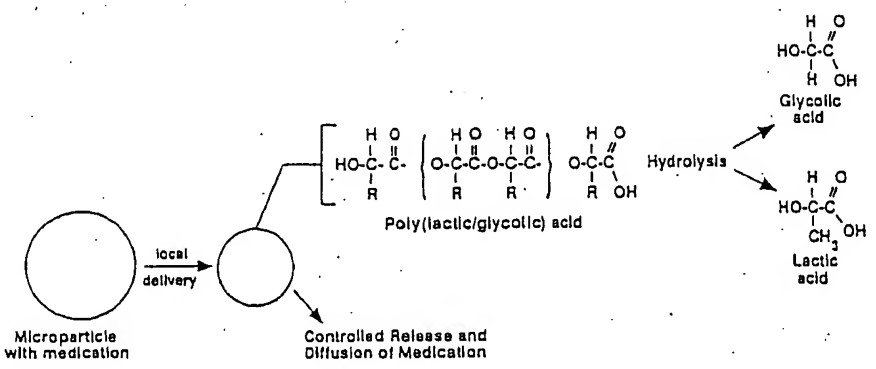
Controlled Release

The local concentration and rate of release of medication can be manipulated by incorporating the agent into a polymeric matrix and depositing the latter locally by catheter-based or surgical techniques. Although many polymers are available for use, biocompatible, biodegradable polymers include polyesters (polylactide, polyglycolide, polycaprolactone, or copolymers), polyalkylcyanoacrylates, polyorthoesters, and polyanhydrides (Ranade 1990). Other biodegradable matrices include various proteins (albumin, gelatin, and zein) or polysaccharides (dextrans and starches). Biodegradable materials may be tailored to release their medications continuously or via a timed burst. Thus, the polymer–medication combination can be manipulated to provide desired release properties. The ideal polymer–drug combination should satisfy the following conditions: (a) adequate drug loading, (b) controlled release of the medication, (c) biocompatibility, and (d) biodegradation of the polymer (Figure 3).

Biodegradable microparticles (poly-D,L-lactide-co-glycolide) containing colchicine or a colchicine analogue have been shown to release the drug and inhibit SMC proliferation in vitro (March et al. 1992). Microparticles could be designed to contain medications that prevent thrombus formation after angioplasty, inhibit platelet deposition, reduce smooth muscle proliferation and/or migration, or attenuate extracellular matrix formation. Release of medications could be designed to occur at different times and at different rates. Microparticles can be specifically delivered in and around the arterial wall with a localization that approaches 99% of the delivered dose with the porous balloon-over-stent catheter (Wilensky et al. 1993). Previous studies have shown that latex microparticles delivered in a similar way are retained locally for at least 2 weeks (Wilensky et al. 1991). Thus, the delivery and long-term retention of microparticles in the arterial wall are feasible.

In addition to intramural drug infusion, periadventitial deposition of an agent can exploit local diffusion as the primary mechanism of delivery. Heparin, contained in ethylene-vinyl acetate copolymer matrices and placed in the periadventitial space of arteries immediately following injury, has been shown to reduce neointima formation at 14 days after balloon injury (Edelman et al. 1990). Non-anticoagulant, low molecular weight heparin was equally effective compared with unfractionated heparin when delivered in the periadventitial space, but was ineffective when delivered via an intravenous or subcutaneous route. Similarly, Okada et al. (1989) applied a polyvinyl alcohol–heparin gel surrounded by a silastic shell to the periadventitial space of balloon-injured arteries and noted a significant decrease in neointima formation at 20 days. This method of periadventitial delivery had previously been shown to reduce local thrombus formation in the absence of a

Figure 3. Drug delivery via a biodegradable microparticle. In this example, the microparticle is composed of a mixed polymer, poly(lactic/glycolic) acid. The agent is incorporated into the matrix during preparation of the microparticles. Medication can diffuse out of the microparticles into surrounding tissues. The polymer is degraded via nonenzymatic hydrolysis. The glycolic and lactic acids liberated can be metabolized or excreted.



systemic anticoagulant effect (Okada et al. 1988). Others have demonstrated, in the rat model, a reduction in neointimal proliferation following adventitial deposition of dexamethasone in a silicone polymer vehicle (Villa et al. 1993). Simons et al. (1992) have used periadventitial delivery to inhibit neointima formation in a rat carotid artery injury model by using an antisense oligonucleotide to c-*myb*, a protooncogene essential for cell cycle progression, and hence proliferation, of SMCs (Simons and Rosenberg 1992). In this case, a water-soluble phosphorothioate derivative of the oligonucleotide was dissolved in a Pluronic gel that was deposited around the injured carotid artery. Similar results have been achieved in rabbit iliac arteries undergoing balloon injury and antisense c-*myb* injection via a porous catheter (R.D. Rosenberg personal communication). Although periadventitial implantation by a surgical approach is not clinically relevant in the treatment of angioplasty restenosis, a large proportion of radioactive microparticles delivered endoluminally are deposited outside of the arterial wall in the periadventitia and the overlying muscle layers (Wilensky et al. 1993). Thus, strategies to modulate the arterial wall processes must take into account the effect on the periadventitia, but also may exploit the resulting periadventitial depositions and luminally directed diffusion of the deposited agent.

Affinity-Based Delivery

One advantage of an affinity-based delivery system is that the agent can be administered systemically (for instance, by oral or intravenous routes) and subsequently accumulate at the site of angioplasty or atherosclerosis. Local accumulation of a synthetic peptide, consisting of amino acid residues 1000–1016 of apolipoprotein (apo) B-100, has been demonstrated at the edges of areas of regenerating endothelial cells 4 weeks following balloon catheter-induced endothelial denudation (Shih et al. 1990). Local uptake of the peptide, visualized by γ scintigraphy, was noted as early as 4½ h after intravenous injection, and persistent accumulation was observed 24 h after injection when plasma levels decreased to <1% of the injected dose. The apoB synthetic peptide bound only to the extracellular matrix of the regenerating endothelium.

Antibodies are among the most specific and high-affinity carriers that might be used for drug delivery. Tissue-type plasminogen activator (tPA) has been covalently linked to a monoclonal antibody directed against fibrin, resulting in increased fibrinolytic activity due to enhanced local plasminogen activation (Schnee et al. 1987).

A major limitation of antibody-based drug delivery systems is the rapid metabolism of the antibodies by the reticuloendothelial system. Antibodies are immunogenic and do not penetrate an intact endothelial barrier. Moreover, no vascular injury-specific antigens have yet been identified as targets for drug carrier antibodies. On the other hand, advances in genetic engineering techniques have made it possible to design chimeric antibodies consisting of hybrid gene segments (Neuberger et al. 1985) combining murine light chains with human heavy chains to enhance efficacy while reducing antigenicity (Hamblin et al. 1987, LoBuglio et al. 1989). In the future, chimeric antibodies may be constructed to carry drugs or other therapeutic agents to endothelial cells, SMCs in either the contractile or proliferating phenotype, or to the extracellular matrix of the vascular wall.

STRATEGIES TO MODULATE THE ARTERIAL WALL PROCESSES MUST TAKE INTO ACCOUNT THE EFFECT ON THE PERIADVENTITIA, BUT ALSO MAY EXPLOIT THE RESULTING PERIADVENTITIAL DEPOSITIONS.

In another affinity-based strategy, therapeutic molecules have been linked to growth factors. SMCs in the synthetic, proliferating phenotype show a tenfold increase in the expression of epidermal growth factor (EGF) receptors compared with SMCs in the contractile phenotype (Epstein et al. 1991). Transforming growth factor alpha (TGF-α) exhibits homology to EGF and binds to the EGF receptor (Todaro et al. 1980). PE40 is an ADP-ribosyl transferase derived from *Pseudomonas* exotoxin (PE) that is internalized and inhibits protein synthesis. A chimeric protein, TGF-α–PE40, was produced by expression of a fusion gene and shown to kill SMCs selectively in culture (Epstein et al. 1991). Cytotoxicity was tenfold higher in proliferating cells than in nonproliferating SMCs. Upregulation of bFGF (basic fibroblast growth factor) receptors similarly occurs in proliferating smooth muscle cells, and a chimeric toxin has also been constructed combining bFGF with the ribosomal protein synthesis inhibitor, saporin. A single intravenous dose of bFGF–saporin (40 μg/kg) given 1–2 days following aortic crush injury inhibited arterial wall DNA synthesis and intimal thickening (Casscells et al. 1990). A subsequent study (Biro et al. 1991) showed a similar effect on rat aortic SMCs following balloon injury and demonstrated a dosing range in which bFGF–saporin was cytotoxic to SMCs but stimulated the proliferation of endothelial cells. Others have demonstrated similar results showing that the bFGF–saporin conjugate reduced the number of medial SMCs in balloon-injured arteries but not in the control, noninjured arteries (Lindner et al. 1991). In another scheme, a chimeric toxin consisting of the ligand aFGF (acidic FGF) fused with a mutant PE significantly decreased protein synthesis of proliferating SMCs in culture, killing 32% of the cells at 48 h (Biro et al. 1992). The cytotoxicity was mediated by specific binding to the FGF receptor and was significantly greater in proliferating SMCs. Since EGF and FGF receptor expression is not specific to proliferating SMCs, systemic toxicity may occur.

Gene Therapy

The introduction of genes into cells of the arterial wall is a strategy that holds promise for future treatment of vascular disease. Already, the feasibility of injecting retroviruses (Nabel et al. 1990, Flugelman et al. 1992), plasmid–liposome complexes (Nabel et al. 1990, Leclerc et al. 1992, Chapman et al. 1992), and DNA without a carrier (Chapman et al. 1992) has been demonstrated using catheter-based technology. For example, Nabel et al. (1990) demonstrated that a β-galactosidase gene in a retroviral vector could be introduced into arterial cells after intramural infusion via a double-balloon catheter. Expression was also obtained with the use of plasmids incorporated into liposomes. β-Galactosidase expression was observed in all layers of the arterial wall. Flugelman et al. (1992) attempted to reproduce these results by

using a porous balloon catheter, and observed increased β-galactosidase staining in eight of nine experimental arteries. However, β-galactosidase activity was also observed in six of nine control arteries. Analysis of the efficiency of transfection at the level of DNA indicated that <100 cells were transfected in a 2-cm segment of injected aorta. In another approach, Lim et al. (1991) used luciferase as a reporter gene to demonstrate transfection in canine left anterior descending and femoral arteries. Luciferase activity was observed for up to 3 days after gene transfer. Leclerc et al. (1992) used liposome-associated plasmids containing the luciferase gene to demonstrate transfection of atherosclerotic rabbit iliac arteries. Furthermore, Lynch et al. (1992) infused nonatherosclerotic rat carotid arteries with retroviral vectors encoding either an *Escherichia coli* β-galactosidase or a human adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) gene. Although no increase in β-galactosidase activity was observed, low levels of ADA gene expression were. Finally, DNA alone in the absence of viral or liposomal packaging can be successfully transfected into arterial wall cells. Chapman et al. (1992) injected luciferase cDNA into canine coronary arteries. Luciferase activity above background was noted in eight of 12 infused coronary arteries 3–5 days after infusion. Transfection efficiency remains low and may limit gene therapy. However, replication-deficient adenovirus vectors recently have been shown to exhibit a high transfection efficiency in endothelial cells of sheep carotid arteries and jugular veins (Lemarchand et al. 1993). In contradistinction to retrovirus vectors, target cell proliferation is not necessary. Insertional mutagenesis does not occur, because the adenovirus is not integrated in the target cell genome. An alternative form of gene therapy is the use of antisense oligonucleotides. These are short segments of DNA or RNA that bind to their complementary mRNA and inhibit translation. Oligonucleotides are transported into cells via size-dependent receptor-mediated endocytosis mechanisms (Loke et al. 1989). The ideal oligonucleotide should possess selectivity, stability, and water solubility (Stein and Cohen 1988) and be nuclease resistant (Cohen 1989, Eckstein and Gish 1989). Specific antisense oligonucleotides can be designed to inhibit synthesis of protein necessary for cellular proliferation or migration and accordingly may be directed toward many possible targets for intervention in the process of restenosis.

An 18-base antisense oligodeoxynucleotide complementary to the mRNA of proliferating cell nuclear antigen (PCNA), an essential cofactor for DNA polymerase δ (Prelich and Stillman 1988), reduces proliferation of rat vascular SMCs in culture (Speir and Epstein 1992). Two other targets for antisense oligonucleotides in SMCs have also been reported: the protooncogene, c-*myb*, and the heavy chain of nonmuscle myosin. c-*Myb* induces DNA polymerase α (Venturelli et al. 1990), PCNA, and histone H3 mRNAs by a posttranscriptional mechanism allowing cellular entry into S phase (Travali et al. 1991). Low levels of c-*myb* mRNA are observed in quiescent SMCs, but increased levels are noted 4–8 h after serum stimulation and reach a maximal level at 12 h (Reilly et al. 1989). Nonmuscle myosin is required for cellular migration and cell division. An 18-base antisense phosphorothioate oligonucleotide to c-*myb* and a second 18-base antisense oligonucleotide to nonmuscle myosin showed antiproliferative effects on SMCs in culture (Simons and Rosenberg 1992). As with antisense PCNA (Speir and Epstein 1992), the effect was reversible following washout of oligonucleotide from the cell culture.

A third strategy for gene therapy involves the local implantation of genetically engineered cells. Gene transfer to the arterial wall has been accomplished with this approach by means of percutaneous catheter injection of transfected endothelial cells (Nabel et al. 1989) and SMCs (Plautz et al. 1991) as well as by implantation of vascular grafts (Wilson et al. 1989) or intraluminal stents (Dichek et al. 1990) coated with transfected endothelial cells. Intravenous infusion has also been used to introduce rat pulmonary endothelial cells transfected with a human growth hormone fusion gene with subsequent production of high levels of the hormone (Bernstein et al. 1990).

Despite the excitement about gene therapy of the arterial wall, many challenges remain to be addressed to establish the utility of this approach. Most studies to date have used reporter genes that have no intrinsic therapeutic value. Unfortunately, a specific therapeutic gene product has not yet been identified. In addition, there is no specificity of targeting (that is, to fibroblasts, endothelial cells, or SMCs). Local implantation of endothelial cells may show particular promise if a specific molecule can be identified that exerts therapeutic effects when secreted locally. However, the need to harvest, transfect, and deliver isologous cells may prove to be logistically impractical in the setting of acute ischemic heart disease. Perhaps the most practical form of gene therapy would be the use of antisense oligonucleotides. These could be modified for sustained local effect by incorporation into microparticles and delivery via a local delivery catheter.

• Conclusion

Local drug delivery offers considerable promise for treatment of restenosis following angioplasty. Although no studies have yet demonstrated clinical efficacy of local delivery, specific devices have been fashioned and key parameters that define many of the technical problems have been documented. The current multidisciplinary approach to solving the problem of restenosis offers genuine hope that prevention is not far away. Moreover, these ongoing investigations raise the possibility of greatly reducing the need for surgical treatment of coronary and peripheral atherosclerosis by highly effective angioplasty procedures combined with rational molecular strategies.

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